CONDITIONS FOR THE GERMINATION OF THE SPORES OF BRYOPHYTES AND PTERIDOPHYTES.

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(WITH PLATE IV)

I. INTRODUCTION.

THE investigations upon the effect of light on the germination of fern and moss spores have led to opposite and contradictory results. According to Borodin, Schmidt and others, the failure of fern spores to germinate in the dark is experimentally demonstrated, while Göppert and Schelting arrived at exactly opposite conclusions. Leitgeb has shown the necessity of light for the germination of liverwort spores, and Milde succeeded in germinating Equisetum spores in the dark. Up to this time no systematic work on the germination of moss spores in light and darkness has been carried out. In order to clear up this existing confusion and extend our knowledge in regard to the conditions for the germination of moss spores, the present investigation was undertaken.

Before proceeding with the results of my own experiments however, I will treat a little more in detail the investigations bearing upon this subject, which have been hitherto published.

II. HISTORICAL.

The early botanists were in no sense of the word physiologists, and so from the time when the spores of mosses were first observed and compared to the seeds of flowering plants nearly to the present time, their germination has been treated almost exclusively from the morphological point of view. A historical summary of the works on the germination of the spores of Musci and Hepaticæ, up to 1884, is brought together by Lind-

berg.[1] The summary is not quite complete, as no mention is made of the result which Borodin[2] obtained with spores of *Polytrichum commune*. He found that they were unable to germinate in darkness. The work of Müller-Turgau[3] on the germination of spores and the production of secondary protonema is also omitted.

As regards the fern spores, the earlier investigators made the assertion that light prevents their germination, as is to be noted in the works of Senebier, Humboldt,[4] Ingenhouss,[5] and Treviranus.[6] More recent investigators, as Kaulfuss,[7] Leszczye-Suminski,[8] Merklin,[9] Wiegand[10] and Hofmeister,[11] intimate that light is one of the necessary conditions for germination, although no definite investigations in that line are mentioned.

The first investigations of importance from the physiological point of view are those of Borodin.[12] He experimented with eight different species of ferns and found that in all cases light was necessary for germination, and that in the dark no bursting of the exine occurred. His experiments are lacking in one datum, since he does not state at what temperature the cultures were kept. As shown by my own investigations this is one of the most important points. Two years later Göppert[13] succeeded in bringing the spores of Osmunda to germinate in the dark, but the temperature at which the cultures were grown is unknown to me. A year later, Schmidt,[14] with cultures of the spores of *Aspidium violaceus* and *filix-mas*, confirmed the results previously

[1] Historiska Data rörande vår Kännedom om Moss-sporens Groning. Helsingfors, 1884. Rectorprogram.

[2] Bull. de l'acad. imp. de S. Petersbourg, **12**: 433–440. 1867.

[3] Arb. d. Bot. Inst. zu Würzburg **1**: 475–499.

[4] Aphorismen 90.

[5] Versuche mit Pflanzen II. 5. Abschnitt.

[6] Physiologie der Gewächse **2**[2]: 584. 1838.

[7] Das Wesen der Farnkräuter 59. 1827.

[8] Zur Entwickelungsgeschichte der Farnkräuter 8. 1849. Berlin.

[9] Beobachtungen am Prothalium der Farnkräuter 5. 1850.

[10] Entwickelungsgeschichte der Farnkräuter. Bot. Zeitung **7**: 17. 1849.

[11] Vergleichende Untersuchungen 78. 1851.

[12] *Ibid*, 529–541.

[13] Schmidt, Über einige Wirkungen des Lichtes auf Pflanzen 21. 1870. Breslau.

[14] *Ibid*, p. 20.

obtained by Borodin. In 1872 Kny[15] obtained results which contradicted those of Göppert for Osmunda spores. The next work of importance was that of Schelting[16] in 1875. He investigated the spores of four different species and found that in all cases germination occurred in the dark. One of the species which he used, *Aneimia Phyllitidis*, was also used by Borodin in the investigations above cited. I have not had access to the original paper, but the probability is, from the review, that the cultures were kept at a temperature higher than the normal room temperature.

Again later, G. Beck[17] has shown that the spores of *Scolopendrium vulgare* germinate only when exposed to light.

Milde[18] and Sadebeck[19] have shown that the spores of Equisetum germinate in the dark as well as in the light; while Leitgeb[20] in his excellent work on the liverworts has shown that darkness prevents the germination of the spores; also that faint illumination causes the development of protonemata which differ markedly in form from those grown under normal illumination. With this short historical summary as an introduction I proceed to the results of my own investigations.

III. EXPERIMENTAL.

The majority of the cultures were made either upon filter-paper, pieces of flower pots, or earth placed in Petri dishes and carefully sterilized. Any special methods will be described in connection with the experiments themselves.

I. MOSS SPORES.

First as to the experiments with moss spores. Cultures of *Funaria hygrometrica* spores were made, and in one case exposed to normal illumination, and in another placed in the dark cham-

[15] Jahrb. f. wiss. Bot. **8**: 4. 1872.
[16] Bot. Jahresber. **3**: 328. 1875.
[17] Bot. Zeitung **36**: 780. 1878.
[18] Nova Acta Acad. L. C. F. **23**: 2.
[19] Bot. Zeitung **35**: 44, 45. 1877.
[20] Sitzungsber. d Akad. d. Wiss. Wien. **74**: 1. 1876.

ber; both being kept at a temperature varying from 19–21° C. At the end of three days the spores exposed to light had germinated abundantly, while those in the dark chamber showed no signs of germination, not even a bursting of the exospore. The dark culture was kept for a month, and at the end of that time there was no indication of germination. That the spores had remained normal and had not lost their power of growth was shown by their speedy germination when exposed to ordinary illumination. This experiment was repeated several times with the same result. Similar experiments were carried out with spores of *Brachythecium rutabulum*, *Bryum pendulum*, and *Mnium cuspidatum*, and all revealed the same dependence of germination upon illumination.

In order to determine which part of the spectrum was effective in producing germination, cultures of spores were placed under double-walled bell-glasses filled respectively with potassium bichromate and ammoniated copper oxide. The cultures included *Funaria hygrometrica*, *Bryum pendulum*, and *Brachythecium rutabulum*. At the end of three days the spores exposed to the less refrangible rays of the spectrum had germinated with as great readiness as under normal illumination, while the cultures in the blue light showed no signs of germination, thus acting the same as in darkness. The spores, although retained in the blue light for over a month, showed no germination whatever. The failure of the spores to germinate in the strongly refrangible rays would seem to throw some light upon the processes which occur in germination. Although the spores form some chlorophyll in the blue light, the photosyntactic processes are not active, and hence it might be thought that germination depended upon the elaboration of new material which can only occur to any extent in the less refrangible rays. That this view is highly improbable is shown by the experiments which follow.

Cultures of spores were made of the three species above mentioned and placed in the light in air free from CO_2, the apparatus being the same as that figured by Pfeffer.[21] The first

[21] Pflanzenphysiologie 1: 191. 1881.

series of experiments in bright light showed that photosyntax was not necessary for germination, since the spores had germinated as readily in the air free from CO_2 as under ordinary conditions. The same result was obtained when the apparatus was exposed to less intense illumination by the interposition of an opaque screen. Under these conditions the photosyntax would be insignificant, and hence it appears evident that germination is independent of that process. The influence of light in germination must be sought, then, presumably in a transformation of food products already present in the spore; these chemical changes being initiated by light, and only by the less refrangible rays. More in regard to the nature of this transformation will be brought forward when later experiments are described.

The question which now presented itself was: Is continued exposure to light necessary to germination; in other words, is there a light induction? In order to determine this point, cultures of spores which had been in the dark for twelve hours were placed in the light and carefully watched for the first signs of germination. After about fourteen hours of illumination the spores showed the first signs of germination, in some cases the exine being burst; a slight protuberance, the beginning of the protonema, was also evident. Half of the cultures were allowed to remain in the light as control experiments, while the other half were removed to the dark chamber to undergo further development. Those spores which showed no beginning of germination before removed to the dark did not germinate in the dark, although they had formed an abundant chlorophyll content. Those spores which had begun to put out a protonemal filament continued their growth somewhat, but the filament was long and slender and did not attain any considerable size on account of lack of plastic material. These experiments were carried out with spores of *Funaria hygrometrica*, *Bryum pendulum*, and *Brachythecium rutabulum*, all with the same result. Essentially the same fact has been shown by Borodin[22] for fern spores.

[22] *Ibid.*, 539.

Leitgeb[23] has shown that for the germination of liverwort spores a certain intensity of light is necessary, and my experiments with moss spores show that the same thing is true, only to a less marked extent. In experiments which I conducted with *Marchantia polymorpha* spores, parallel with the cultures of moss spores, where all were exposed to the light in the middle of the laboratory, the different behavior was very marked. The moss spores germinated in the usual length of time and without any apparent modification due to the weakness of the light. The Marchantia spores, on the other hand, showed a very remarkable retardation in germination, and when germination did occur only a long, narrow filament was produced, which gave no indication of the formation of the thallus according to the ordinary method of growth in sufficiently intense illumination. Other cultures were made and exposed to a much weaker light, a room in the Institute basement with only one window being used. First, cultures were placed on a shelf at a distance of about three meters from the window, then at two meters, and then in the window itself. The cultures at a distance of three and two meters from the window showed a complete failure of the spores to germinate, although they produced chlorophyll to some extent. Those which were grown in the window germinated after the ordinary length of time. That the spores remained capable of germination was shown by the fact that they began growth as soon as they were exposed to normal illumination. These experiments then show that under ordinary conditions of temperature and food supply, the moss spores require a certain intensity of light for germination, but that the required intensity is not as great as in the case of the liverwort spores. These facts were demonstrated for the three species of mosses mentioned above.

Reasoning from the results which I had already obtained with fern spores, a series of experiments was carried out in which cultures of spores were exposed to different temperatures. The failure of the spores to germinate in the dark is due, as has

[23] *Loc. cit.*

already been stated, to the fact that the conditions of temperature, light, etc., were such that certain chemical processes necessary for germination could not be active. The results with fern spores show that heat is able to effect this change as well as light, so that germination may be called forth in complete darkness by subjecting the spores to a higher degree of temperature than the normal room temperature. That moss spores would be affected in the same way as fern spores would seem quite probable, but nevertheless my experiments in this direction have failed to find any temperature at which moss spores will germinate in complete darkness, when supplied with only inorganic material. The temperature to which the general cultures were exposed ranged from 19–21° C. Cultures were made for the following degrees of temperature: 23°, 24°, 27°, 29°, 32°, and 35° C., and in each case in complete darkness. The cultures at 35° C. were kept for four days. At the end of that time no signs of germination were visible, hence they were removed to the light and kept at the ordinary temperature. The failure of the spores to germinate under these conditions showed that they had been killed by the high temperature. The other cultures were allowed to remain in the dark for six days and then removed to the light. The spores subjected to 32° were not killed, but a very marked retardation of germination occurred, since the period required for germination was extended from three days to ten. The cultures that had been kept at 29° also showed a retardation of germination, five days being required after the exposure to light. In the other three series of cultures at 27°, 24°, and 23°, no apparent retardation of germination was noticed when the spores were exposed to light.

The above experiments have shown clearly that a continuous exposure to high temperatures is not sufficient to produce germination of the moss spores in the dark. As suggested by the results obtained by Liebenberg[24] for seeds of Poa, it was thought that perhaps a *change* of temperature might be effective in producing germination. To this end the following experi-

[24] Bot. Centralblatt **14**: 21–26. 1884.

ments were carried out: Two cultures of *Funaria hygrometrica* spores were placed in the dark for twelve hours, then in the thermostat at 41° C. for four hours. The cultures were then removed and one placed in light, the other in the dark, both at a temperature of 19–21° C. After three days the spores in the light had germinated abundantly, but those in the dark showed no signs of growth, although they were kept for two weeks. Similar cultures were exposed to a temperature of 41° C. for three hours with the same result.

Cultures similar to the above were made for *Funaria hygrometrica*, *Bryum pendulum*, and *Brachythecium rutabulum* and exposed to a temperature of 32° C. for twenty-four hours. At the end of this time they were placed at the ordinary temperature, the control experiments in the light, the others in the dark. Those in the light germinated after the usual length of time, but in the dark no signs whatever of germination were noted. Thus, change of temperature is also shown to be insufficient in producing germination in complete darkness.

It is known that ether has a stimulating influence on the production of shoots from certain phanerogams, when under normal conditions none are produced. It might also be supposed that it would act as a stimulus to call forth the germination of spores in the dark. In order to determine this point, a series of cultures was made in which the spores were subjected for different lengths of time to a saturated or partially saturated atmosphere of ether. Cultures of Funaria spores were allowed to remain in the dark for twenty-four hours, in order that they might be in a moist condition, and then placed in an ether atmosphere. In the first case they were exposed to the ether atmosphere for one hour, in the next for two hours, and in the next for three hours. Two cultures were used in each case and as soon as they were removed from the ether atmosphere, one was placed in the light and the other in the dark chamber. The control experiments in the light showed, in the experiments to which a two and three hours' exposure to ether was given, that germination did not take place, and hence that the spores had

been killed by the strong ether atmosphere. In the case of spores which had been in the ether for one hour, germination occurred in the light, but it was considerably retarded. In the dark no sign of germination was noted.

From the above experiments it was quite evident that too strong a dose of anæsthetic was administered. Another series of experiments was therefore conducted in which the spores were subjected to an atmosphere containing less ether. In order to supply the ether atmosphere, one part of ether was mixed with twenty of water. The cultures were then exposed to this atmosphere for one and three hours respectively. Those spores which had been in the ether atmosphere for one hour showed a very slight retardation of germination even in the light, but in the corresponding dark culture no germination whatever was noted. Those spores which had been in the ether atmosphere for three hours showed a very marked retardation, the period required for germination being extended from three to ten days. Those in the dark showed no germination. Another culture was treated in a slightly different way; it was placed in the ether atmosphere for one hour, then in the dark for twenty-four hours, then in the ether atmosphere again for one hour, and from that time on in the dark under ordinary conditions. These cultures were kept in the dark for three weeks with the complete failure of the spores to germinate. In so far as the above experiments are concerned, ether retarded the germination of the spores even in the light, and had no effect upon their germination in the dark.

The non-nitrogenous food supply of spores is in the form of oils or fat. The first change of the fats in germination is apparently a decomposition into glycerin and fatty acid.[25] That the ultimate product from this food supply which is used in the first growth is a carbohydrate in the form of sugar may be surmised. At any rate the failure of the spores to germinate in the dark is due presumably to the fact that conditions are not afforded for the chemical changes which the reserve material

[25] Vines, Physiology of plants 173. 1886.

must undergo before it can be used as plastic material for the growth of the cell. There is a possibility that the failure to germinate may be due to the proteid reserve material remaining in a form which cannot be used. This is, however, not as probable as the view just advanced for the non-nitrogenous reserve food. If the supposition is correct, spores when supplied with organic material in the form which the reserve assumes ultimately in germination might be expected to germinate in complete darkness.

As a nutritive solution the following preparation was made: To 100^{cc} of 0.25 pro mille normal inorganic nutritive solution, 2 per cent. of grape sugar and 1 per cent. of peptone was added, and the whole sterilized on the water bath for one hour. Cultures of Funaria spores were made for both light and dark and supplied with this nutritive solution, as great precautions as possible being taken to keep the cultures sterile. An examination of the cultures at the end of three days showed that the spores had germinated as well in the dark as in the light. The very noticeable feature of this experiment was that under these conditions the protonemata were four or five times as large as when supplied with only inorganic nourishment and grown in the light; also that the cells were crowded with large, irregular starch masses, as shown by the iodine test (see *fig. 2*). The question now was: Is this germination in the dark due to the sugar or the peptone or both? In order to determine this point, the following experiments were carried out:

A 2 per cent. grape sugar solution was made from the 0.25 pro mille normal nutritive solution, and cultures of the Funaria spores made for both light and dark. After three days an examination of the cultures showed that germination had occurred as well in the dark as in the light, thus demonstrating the power of grape sugar alone to call forth germinations in the dark. A 1 per cent. peptone solution was then prepared in the 0.25 pro mille normal nutritive solution, and cultures of Funaria spores made for both light and dark. After three days, these cultures also showed germination of the spores in both light and dark-

ness, with the same increase in size of the protonema as in the case where sugar alone was used. In the cultures with sugar nearly every spore germinated, both in light and in darkness; in the peptone culture in the light also the same, but in the dark the number of spores which germinated was relatively small. The above results had already been obtained when Goebel's preliminary note[26] concerning the same phenomenon appeared.

Similar experiments to the above were carried out for *Bryum pendulum*, *Brachythecium rutabulum*, and *Mnium cuspidatum*. With these species essentially the same results were obtained as regards the germination in light and darkness, but the protonemata showed no increase in size, which was such a noticeable feature in the case of the Funaria spores. In the peptone cultures the number of spores germinating in the dark was rather smaller than for Funaria.

That the germination in the dark is due to the nutritive value of the sugar and peptone is highly probable, but still it might be claimed that osmotic pressure was the active agent. In order to throw some light upon this point, the following experiments were carried out: Spores of *Funaria hygrometrica*, *Bryum pendulum*, and *Brachythecium rutabulum* were placed in culture and supplied with a 0.5 per cent. solution of KNO_3. The cultures in the light showed germination after the usual length of time, but no sign of germination was observed in those which were deprived of light. Experiments with the same results were also carried out for the same species in a 1 per cent. solution of KNO_3. De Vries[27] has shown that the osmotic value of KNO_3 is about double that of grape sugar with equal parts of the gram-molecule, or more exactly, the isotonic coefficient of grape sugar is 1.88. The osmotic value of a 0.5 per cent. solution of KNO_3, or approximately a $\frac{1}{6}$ gram molecule solution, would be about the same as that of a 2 per cent. grape sugar solution, or a $\frac{1}{9}$ gram-molecule. The failure then of the spores to germinate under the above conditions would tend to show that the osmotic pres-

[26] Flora **82**: 75. 1896.

[27] Jahrbücher für wiss. Bot. **14**: 454. 1884.

sure within the spore could not have been the operative force in bringing about germination

The absence of any effect from osmotic pressure was also rendered probable from the experiments in which the spores were supplied with either glycerin or potassium tartrate. Spores of the three species above mentioned germinated readily in 1 and 2 per cent. solutions of glycerin in the light, but in the dark they remained unchanged. Glycerin is a non-nutritive substance for the moss-spores, and at the strength used would be about osmotically equivalent to the sugar. In the 1 per cent. solution of potassium tartrate the spores germinated neither in the light nor dark, but in the 0.5 per cent. solution the growth was the same in the light as in the control experiment. In the dark cultures supplied with 0.5 per ccnt. potassium tartrate there was a complete failure to germinate. The isotonic coefficient of potassium tartrate is 3.99[28], and consequently the osmotic value of the last solution would not be far from that of 2 per cent. grape sugar. Cultures of spores which were supplied with a 2 per cent. solution of lactose, also non-nutritive for the moss spores, showed the same failure of germination in complete darkness. The spores which were exposed to light germinated however with as great readiness as in the control experiment, where they were exposed to ordinary conditions.

It is known that certain substances like iron chloride and cobalt salts, when used in a solution which is too dilute to be poisonous, exercise an accelerating influence upon the growth of fungi. The substances are non-nutritive, and the acceleration of growth is presumably due to a so-called catalytic action. This fact suggested the possibility of calling forth germination in the dark by means of such substances, and to this end the following experiments were performed: Spores of the species generally used were grown in different strengths of iron chloride: 0.25 per cent., 0.125 per cent. In no case was germination called forth in the dark. In the 0.25 per cent. solution the Bryum and Brachythecium spores germinated neither in light

[28] DE VRIES, *Ibid.* 506.

nor darkness. In the 0.125 per cent. solution, however, the spores germinated abundantly in the light. A series of experiments was also carried out in which the spores of *Funaria hygrometrica* were supplied with a dilute solution of cobalt sulfate, $CoSO_4$. I have shown in my investigations with seedlings[29] that cobalt solutions are extremely poisonous; hence in order to obtain solutions which would not have a toxic action, a very great dilution of the stock solution was required. Sowings of the spores were made for both light and darkness and supplied with $\frac{1}{10000}$, $\frac{1}{20000}$, $\frac{1}{40000}$, and $\frac{1}{80000}$ gram-molecule solutions. In all of the cultures the spores germinated in the light without any marked retardation, but in the dark, the same as in previous experiments, no germination occurred. Thus all of the previous experiments point to the fact that germination in the dark was due to the nutritive value of the sugar and peptone, and not to any stimulating or catalytic action.

It is also interesting to know the minimum amount of sugar which will suffice to call forth germination in the dark. First, cultures of Funaria spores were supplied with $\frac{1}{900}$, $\frac{1}{225}$ and $\frac{1}{135}$ gram-molecule solution of grape sugar and placed in darkness. An examination after three days showed that in the first two dilutions, none of the spores had germinated, while in the $\frac{1}{135}$ gram-molecule solution they had germinated the same as in light under ordinary conditions; and also with the usual increase in size, and with the accumulation of starch. The spores of *Bryum pendulum* also germinated in a solution of the same dilution, but those of Brachythecium required a still stronger solution, only germinating in the dark when they were supplied with $\frac{1}{90}$ gram-molecule. The maximum concentration at which germination can occur is not so important, but results were obtained in this line for a single species. Cultures of Funaria spore were supplied with 5, 10 and 20 per cent. solutions of grape sugar. The first two concentrations allowed germination in both darkness and light, but in the 20 per cent. solution the spores germinated neither in light nor darkness. In the 5 and 10 per

[29] BOT. GAZ. **22**: 143. 1896.

cent. solutions, the protonemata which were formed in the light were perfectly colorless and without chlorophyll.

The great difficulty of obtaining perfectly sterile cultures of moss protonemata upon an organic substratum will at once be evident to all who have ever worked in this line. Goebel[30] was unable to obtain perfectly sterile cultures in his investigations upon *Funaria hygrometrica*. If perfectly sterile cultures could be obtained, it would be possible then to determine whether moss protonemata are able to thrive in the dark, when supplied with organic material, as sugar and peptone. This was the problem which now presented itself for solution and to which my attention was next directed. A considerable number of attempts were made, and at last my efforts met with success. The details of the experiments I will describe in the order in which they were carried out.

The medium for the growth of the spores was made as follows: 200^{cc} of 0.23 pro mille normal nutritive solution; 2 gr. grape sugar; 1 gr. peptone; 1 gr. agar-agar. The mixture was boiled on a water bath for three hours and then filtered, and preparations made in small Petri dishes.

Capsules of Funaria were selected which had the opercula still intact and attempts were made to sterilize them. They were first soaked in water until the water had penetrated them thoroughly and then placed in 1 per cent. formol for different lengths of time. The preparation of the cultures was carried out under all possible precautions against infection, in a chamber which had been saturated with steam. So far as these experiments were concerned it was shown that an immersion in the 1 per cent. formol for a length of time sufficient to kill the adhering germs, also proved fatal to the spores.

In order to preserve the spores and at the same time have the capsules in a sterile condition, I was obliged to resort to another method. The capsules were first dipped in melted paraffin and perfectly coated over, and then placed in the formol. The coating of paraffin thus prevented all penetration of the

[30] GOEBEL, Sitzber math.-phys. Classe k. Bayer. Akad. Wiss. **26**: 462. 1897.

formol into the interior of the capsules. Then, by operating in the chamber which had been saturated with steam, perfectly sterile cultures were obtained.

The Petri dish cultures offered subsequent opportunities for the penetration of molds, even when extreme care was taken, so that cultures which had been kept sterile for several weeks would often be spoiled by the inroads of fungi. The best results were obtained with cultures made in Erlenmeyer flasks.

Cultures of Funaria protonemata were kept in Erlenmeyer flasks from the first of January to the first of May, four months, in perfectly sterile conditions, and in both light and darkness. Parallel with these was a culture started at the same time upon sterilized earth. The mode of growth of the protonemata on the earth agreed with that already described by Schimper,[31] two protonemal axes generally being produced from each spore and growing in opposite directions. There was almost a complete absence of any rhizoid production. Müller-Turgau[32] claims a quite abundant production of rhizoids by Funaria protonemata. After growing for nine weeks the protonemata had produced an abundance of buds, and rhizoids were then produced from the basiscopic cell of the bud.

Mention has already been made[33] of the power of *Barbula muralis* protonema to separate into distinct cells, which are conidia-like and have the power of growing into new protonemata. Sachs[34] speaks of this capability in regard to Funaria protonemata, and Schröder[35] states it as a general principle, that moss protonemata, when cultivated on too dry soil, break up into the separate cells, which are more resistant, and grow into new protonemata under favorable conditions. In case of the leaf protonema of *Barbula muralis* I have shown that this manner of growth cannot be due to desiccation, since the culture was sup-

[31] *Ibid., plate 1.*

[32] Arb. Bot. Inst. Würzburg 1:480. 1874.

[33] Gametophytic regeneration as exhibited by mosses 25. Oswald Schmidt, Leipzig. 1897.

[34] Lehrbuch der Bot. 366. 1874.

[35] Unt. Bot. Inst. Tübingen 2:15–21. 1886.

plied with abundant moisture. In my cultures of Funaria protonemata on earth this manner of growth was very marked. The cultures were supplied with a considerable amount of moisture, so that the separation into the individual cells could hardly have been called forth by an insufficient amount. In the original culture the spores were sowed in the center of the Petri dish, and after several weeks of growth only covered an area about 2^{cm} in diameter. After ten weeks nearly the whole Petri dish (6^{cm} in diameter) was filled with a luxuriant growth of protonemata, a large majority of which had grown from separate cells.

The cultures in the light on agar-agar produced no buds although they were exposed to sufficient illumination for four months. The control culture on earth had produced an abundance of buds after nine weeks. The growth of the whole protonema was not as vigorous as in the control experiment. *Fig. 4* shows where the original spore cell has started to form a bud by the insertion of an oblique cross wall; further than this, however, no indication of bud formation was noted. The cultures in the dark produced protonemata of considerable size and vigor, but the vigor of growth was markedly below the same cultures in the light. The protonemata were perfectly free from chlorophyll, and the considerable size attained shows that to a certain extent they are able to adapt themselves to a saprophytic mode of nourishment.

In the light the main protonemal axes were directed parallel to the incident rays of light, and grew either in or on the surface of the culture medium. The secondary branches grew erect from the prostrate axes, and directed themselves towards the light at an angle of about 45°, thus exhibiting a marked positive heliotropism. Sachs[36] has already referred to the so-called dorsiventrality of Funaria protonema. In the dark the main axes were without any definite direction, while the secondary branches grew more or less vertical, but irregularly in all directions. Whether this vertical growth of the secondary branches in the dark is due to negative geotropism, I am not able as yet

[36] Vorlesungen über Pflanzenphysiologie 640. 1882.

to state with certainty. Some cultures which were grown in the dark in an inverted position showed that the protonemal branches grew downward, away from the culture medium. These experiments would tend to show that the vertical growth was not due to geotropic sensitiveness, but rather perhaps to negative hydrotropism, as in the case of fungi. More experiments in this line are necessary, however, to make these conclusions certain.

It was thought that by using the culture medium with less peptone the protonemata might be brought to a more vigorous development. A second series of experiments was carried out, in which the same culture medium as described above was used, except that only traces of peptone were added. Even in this medium the protonemata did not grow with their normal vigor, either in light or darkness. The growth was, however, more luxuriant than in the cultures which were supplied with more peptone.

2. MARCHANTIA SPORES.

The work of Leitgeb upon the effect of light on the germination of liverwort spores has already been mentioned. Since he found that the spores were unable to germinate in complete darkness, a confirmation of his results at this point cannot be without interest. A culture of Marchantia spores was kept in the dark for over two months without any signs of germination. At the end of this time they were placed in the light, and after a lapse of six days the majority of the spores had germinated. My experiments also confirmed his results in regard to the intensity of light necessary for germination. In weak light germination was retarded, and when growth did take place the spores produced only a narrow filament, with a small amount of chlorophyll. The filament did not attain any considerable size or form a germ disk.

As regards the part of the spectrum effective in producing germination, my experiments with Marchantia spores yielded the same results as for moss spores, and the same as has been found

for fern spores by Borodin.[37] That is, the blue rays, the more strongly refrangible, have apparently the same effect as complete darkness. In the potassium bichromate light germination occurred after six or seven days, and with every evidence of as vigorous growth as under normal illumination.

The effect of temperature on the germination of Marchantia spores in the dark was also investigated. A series of cultures was made for 32°, 29°, 27°, 24°, and 23° C., and all placed in the dark. After two weeks' time they were observed, and none of the spores had germinated. They were then placed in the light to see if they had remained capable of germination. In the cultures which had been kept at 29° and 32° C. germination was retarded for a few days, but the spores from the remainder of the cultures grew after the usual length of time.

The length of time required for germination, and the condition of the material, rendered the preparation of sterile cultures impossible, so that the cultures with grape sugar were not very satisfactory. Although I did not succeed in bringing the spores to germinate under these conditions, they gave every indication of growth, and I attribute the failure to germinate only to the inroads of bacteria. In the dark in sugar solution the spores increased to three or four times their original diameter and formed large starch masses the same as in the moss protonemata.

Further than this they could not be brought, although repeated attempts were made, and when the cultures were placed in the light even then germination did not proceed, showing that the spores had not remained capable of germination. If, however, the cultures could have been kept sterile, there was every indication that the spores would have germinated.

3. FERN SPORES.

A culture of the spores of *Ceratopteris thalictroides* was kept in the dark for three months without any signs of germination, while a sowing of the same spores germinated in the light after

[37] *Ibid.*, 536.

twelve days. Experiments with the spores of *Alsophila Loddigesii* led to the same results. Thus for the species investigated, it can be stated as certain, that under ordinary conditions of nourishment and at a temperature of 19–21° C. the spores are not capable of germinating. The effect of a higher temperature was then tried for the spores of *Ceratopteris*, a culture of the spores being kept at 32° C. in the dark. After a lapse of sixteen days the culture was examined, and it was found that the spores had germinated abundantly. The form of growth was that of a cell filament, seven or eight cells long, the whole being about 2^{mm} in length (see *fig.* 5). The basal cell always produced a rhizoid, and in some cases the end cells of the prothallium divided also longitudinally. The comparative size and form of a prothallium grown under these conditions and one grown in light at the normal temperature is shown in *figs.* 5 and 7. Experiments of a similar nature were also carried out for *Alsophila Loddigesii*. These experiments are interesting in that they show how Borodin and Schmidt, and Göppert and Schelting could have obtained such contradictory results.

4. EQUISETUM SPORES.

There are no contradictory views in regard to the germination of Equisetum spores, both investigations cited admitting and establishing the fact that germination occurred in perfect darkness. I have repeated these experiments for spores of *Equisetum arvense*, with the same result. It can then be stated with absolute certainty, that Equisetum spores are able to germinate under ordinary conditions of nourishment and at a temperature of 19–21° C., in darkness as well as in light. From the foregoing results it seems that light or organic nourishment is one of the necessary conditions for the germination of moss and liverwort spores, in order that chemical changes may take place which will bring the reserve food material into a condition in which it can be used in growth. For the ferns, these chemical processes may be initiated either by light or a sufficiently high temperature, while in the case of Equisetum these

changes can occur at a much lower temperature in both light and darkness.

IV. SUMMARY.

The more important results of the foregoing investigations may be stated as follows:—

1. Under ordinary conditions of temperature and inorganic nourishment, moss and liverwort spores are unable to germinate in the dark. Spores when subjected to the more strongly refrangible rays of the spectrum only behave the same as in darkness.

2. Organic nourishment in the form of either peptone or grape sugar will call forth the germination of moss spores in complete darkness. Moss protonemata are able to attain a considerable size in the dark, by a saprophytic nourishment, although the vigor of growth is considerably below the normal.

3. Under ordinary conditions of temperature and inorganic nourishment, fern spores are unable to germinate in the dark. A higher temperature, however, will furnish conditions for the germination in complete darkness.

4. The spores of Equisetum germinate apparently as well in darkness as in light and at the ordinary room temperature of 19–21°C.

The experiments for this work were conducted during 1896–7 at the Botanical Institute, Leipzig, under the direction of Herr Geheimrat Professor Dr. Pfeffer, to whom thanks are due for many valuable suggestions.

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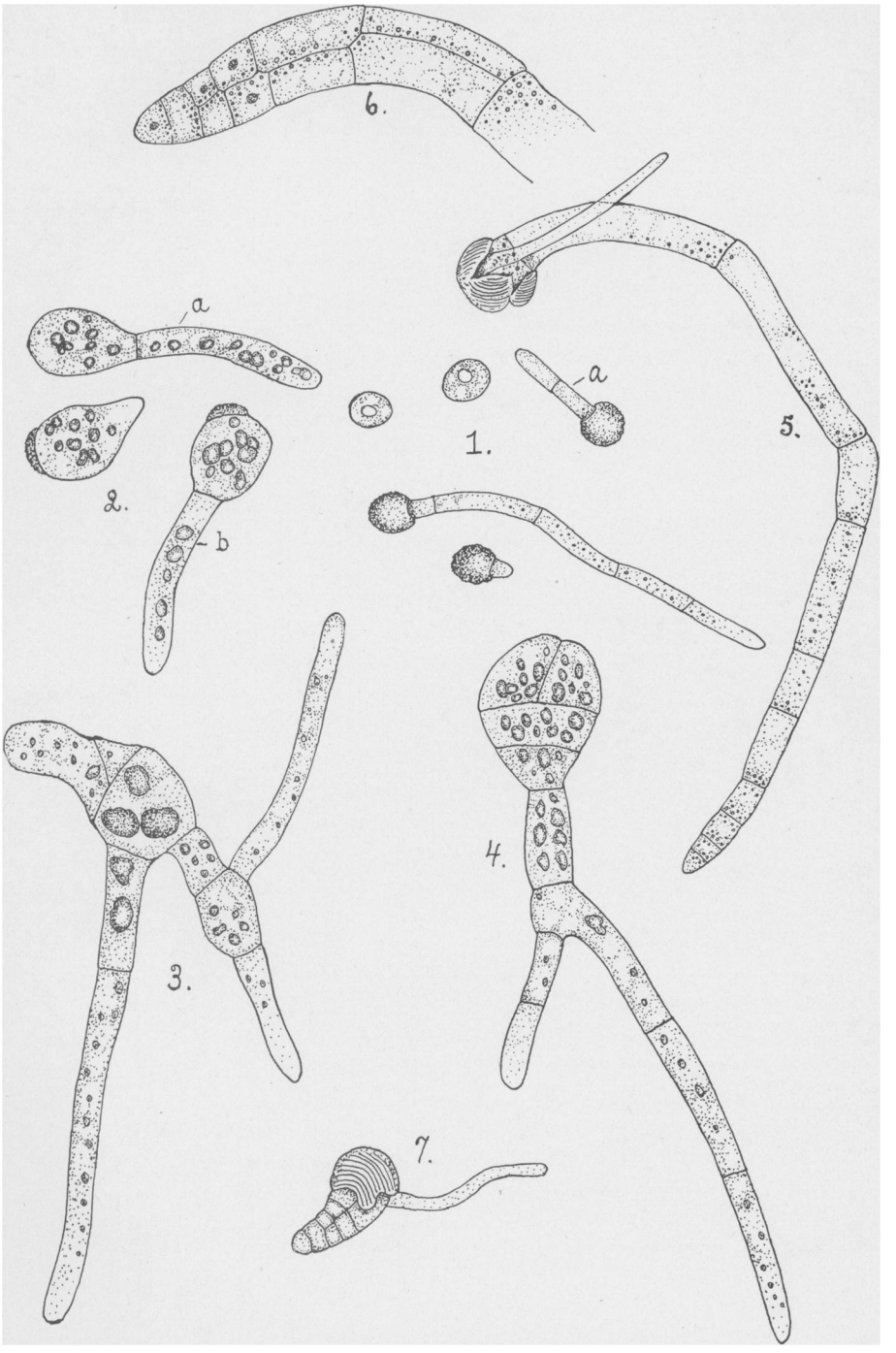
EXPLANATION OF PLATE IV.

FIG. 1. Various stages of Funaria spores, germinated in the light under normal conditions; *a*, after being in culture for three days. × 330.

FIG. 2. Spores of Funaria germinated in the dark in a sugar solution; *a* and *b*, after being in culture for three days. × 330.



HEALD on GERMINATION of SPORES

FIGS. 3, 4. Protonemata of Funaria grown in the dark; eight days on peptone, sugar, and agar-agar. × 330.

FIGS. 2, 3, 4 show a large number of starch masses.

FIG. 5. Prothallium of *Ceratopteris thalictroides*, grown in darkness, at 32°C. In culture sixteen days. × 330.

FIG. 6. Apical portion of a prothallium of the same species, showing longitudinal as well as transverse divisions. × 330.

FIG. 7. Germinating spores of the same age as in *fig. 5*, but grown in light at a temperature of 19°—21°C. × 330.